

I. AMENDMENT

A. In The Specification

Please enter the following rewritten paragraphs:

[0148] Various methods and algorithms have been used for analyzing results and presenting the results in useful form for interpretation. Eisen, et al., Proc. Natl. Acad. Sci. USA (1998) 95, 14863-14868, (specifically incorporated herein by reference) describes using cluster analysis using standard statistical algorithms with graphic display of the results. By employing a dendrogram that assembles all elements into a single tree, where for any set of n genes, an upper diagonal similarity matrix is computed by using the described metric, which contains similarity scores for all pairs of genes. The matrix is scanned to identify the highest value (representing the most similar pair of genes). A node is created joining these two genes, and a gene expression profile is computed for the node by averaging observation for the joined elements (missing values are omitted and the two joined elements are weighted by the number of genes they contain). The similarity matrix is updated with this new node replacing the two joined elements, and the process is repeated n-1 times until only a single element remains. Software implementation of this algorithm can be obtained from the authors on the world wide web at [[http://](http://rana.stanford.edu/clustering)] rana.stanford.edu/clustering.

[0172] Brain soluble extracts were run over a Q sepharose column using an ÄKTA FPLC (Amersham Pharmacia Biotech) and eluted with a linear gradient of 0-500 mM NaCl. Samples of the elution fractions (10 x 2.5 mL fractions) were labeled with FP-biotin as described above, and those fractions containing the 75 kDa and 85 kDa labeled proteins were pooled and passed over a Mono-Q sepharose column. Proteins were eluted from the Mono-Q column with a linear gradient of 200-500 mM NaCl and those elution fractions enriched in the two labeled proteins were then run on SDS-PAGE and transferred to polyvinylidene difluoride

(PVDF) membranes by electroblotting. Regions of the PVDF membranes containing the 75 and 85 kDa FP-biotin reactive proteins were excised, digested with trypsin, and the resulting peptides analyzed by matrix-assisted laser desorption ionization (MALDI) and MALDI-post-source decay time-of-flight mass spectrometry (Chaurand, et al. (1999) J. Am. Soc. Mass. Spectrom. 10, 91-103) on a Kratos Kompact Seq Instrument equipped with a curved field reflectron. The MALDI peptide data were used in MS-Fit and MS-Tag searches of the ProteinProspector databases (found on the world wide web at [<http://falcon.ludwig.ucl.ac.uk/mshome3.2.htm>]), which identified the 75 kDa protein as the rat orthologue of a human protein sequence KIAA0436 and the 85 kDa protein as acylpeptide hydrolase (APH). (see Figure 5).

[0220] Rat liver soluble fractions were run over a Q Sepharose column by using an AKTA FPLC (Amersham Pharmacia Biotech) and eluted with a linear gradient of 0-500 mM NaCl. Aliquots of the elution fractions (10 x 2.5 mL fractions) as well as the flow through (3 x 2.5 mL fractions) were labeled with 1 as described above to identify the fractions containing the labeled proteins. The flow-through fractions, which contained the 55 kDa protein, were concentrated to a volume of 1 mg protein/mL vol. followed by labeling 2.5 mL of the sample with 1 utilizing the standard conditions. After incubating the reaction for thirty minutes, it was applied to a PD-10 size exclusion column and eluted with 3.5 ml of pH 8, 50 mM Tris-HCl buffer. Sodium dodecyl sulfate (SDS) (0.5% wt/vol) was added and the labeled samples heated to 90°C for 10 min in order to denature the proteins allowing for a more accessible biotin moiety. The sample was then diluted 2.5 fold (0.2 % SDS) and incubated with 50-100 µL of avidin beads on a rotator for 1 hour at 25°C. The eluant was then removed followed by washing with 5 ml of 0.2% SDS and three washes with pH 8, 50 mM Tris-HCl buffer. Standard 2x SDS-PAGE loading buffer was added followed by heating the sample to 90°C in order to elute the proteins labeled with 1 from the avidin beads. The eluant was run on an 8% Novex Tris-Glycine gel and stained with Coomassie blue stain followed by destaining in a 30% methanol-water solution. The desired 55 kDa 1-reactive-protein was excised from the gel and digested with trypsin. The resulting peptides were analyzed by matrix assisted laser desorption

In re Application of:
Cravatt et al.
Application No.: 09/738,954
Filed: December 15, 2000
Page 4

PATENT
Attorney Docket No.: SCRIP1210-2

time-of-flight (MALDI-TOF) mass spectrometry. The MALDI peptide data was utilized in both the MS-Fit search of the Protein Prospector databases (falcon.ludwig.ucl.ac.uk/mskome3.2.htm) and the ProFound search of the Proteometrics databases (found on the world wide web at [\[www.\]proteometrics.com/prowl-cgi/ProFound.exe](http://www.proteometrics.com/prowl-cgi/ProFound.exe)), which identified the protein as cytosolic 2 class I rat aldehyde dehydrogenase (cALDH-I).